# Evaluation of antioxidant status of female diabetic patients in Nnamdi Azikiwe University Teaching Hospital, Anambra State, Nigeria.


## ABSTRACT

Diabetes mellitus has become an onerous disease to developing countries such as Nigeria. Rapid acceptance of urbanisation and sedentary life styles pose an encumbrance to its prevention and management. Increased oxidative stress in diabetes mellitus has been implicated as a culprit in perpetuating antioxidant depletion and diabetic complications in diabetes mellitus individuals. This study aims to evaluate the level of antioxidant status in type 2 diabetes mellitus (DM) female participants visiting the out-patient diabetic clinic of Nnamdi Azikiwe University Teaching Hospital (NAUTH), Nnewi, Anambra State, Nigeria. A total of 86 participants aged 51±10 years were recruited for this study. The test group consists of 43 already confirmed type 2 diabetes mellitus females, while the control group consists of 43 apparently healthy females. The test subjects were further subgrouped into good and poor glycaemic control groups, using a cut-off of <7% for HbA1c. Whole blood was collected from participants and aliquoted into specified sample containers for analysis of the following parameters: random blood glucose (RBG; mg/dL), glycosylated haemoglobin (HbA1c; %), glutathione reductase (GR; U/L) and total antioxidant status (TAS; mmol/L). Results from this study showed that the mean differences in RBG (197.74±49.29 mg/dL) and HbA1c (9.86±1.44%) were significantly higher in the test group compared to the control group RBG (104.79±11.33 mg/dL) and HbA1c (5.21±1.23%) ($P< 0.05$). The mean differences of GR (45.81±20.45 U/L) and TAS (1.81±1.04 mmol/L) were significantly lower in the test group compared to the control group GR (61.21±14.34 U/L) and TAS (2.73±2.08 mmol/L) ($P< 0.05$). The poor glycaemic test group was observed to have the highest RBG (200.34±50.4 mg/dL) and HbA1c (10.23±1.33%) compared both to good glycaemic test group RBG (186.38±45.39 mg/dL), HbA1c (6.54±0.45%) and non-diabetic group RBG (104.79±11.33 mg/dL) and HbA1c (5.21±1.23%) ($P<0.05$). Glutathione reductase (40.66±15.48 U/L) and TAS (1.80±1.08 mmol/L) were significantly more depleted in the poor glycaemic test group compared to the non-diabetic group GR (61.21±14.34 U/L), TAS (2.73±2.08 mmol/L) and good glycaemic test group GR (68.38±25.09 U/L), TAS (1.86±0.92 mmol/L) ($P<0.05$). Out of the 43 participants in the test group, only 18.6% had good glycaemic control and 81.4% had poor glycaemic control. There were significant negative correlations between RBG and TAS ($r=–0.260$; $P=0.015$); RBG and GR ($r=–0.403$; $P=0.000$) and HbA1c and GR ($r=–0.471$; $P=0.000$) ($P<0.05$). However, HbA1c and TAS showed no significant correlation ($r=–0.170$; $P=0.119$) ($P>0.05$). This study concludes that there is antioxidant depletion in females with type 2 diabetes.


KEY WORDS: Antioxidants.
Diabetes mellitus.
Glutathione reductase.
Hemoglobin A, glycosylated

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## Introduction

In developing nations including Nigeria, there is an increasing incidence and prevalence of non-communicable diseases such as diabetes mellitus, which is gradually exceeding that of communicable diseases.[1] Rapid urbanisation, sedentary life style and poor feeding habits have been predicted as a leading cause to increase diabetes mellitus prevalence in Africa.[2] Diabetes mellitus (DM) is a group of metabolic diseases resulting from a defect in glucose metabolism, and its prevalence is increasing globally.[3] There are three types of diabetes mellitus: type 1 diabetes mellitus (also known as insulin-dependent diabetes mellitus) and type 2 diabetes mellitus (non insulin dependent diabetes mellitus). The third type is gestational diabetes mellitus, which has its onset during pregnancy.[4] The increasing prevalence of type 2 diabetes mellitus in Nigeria is alarming; with a prevalence of 4.04% in 2011,[5] 4.83% in 2012[6] and 4.99% in 2013.[7]

Antioxidants are substances which when present in low concentrations, compared to their oxidised substrates, can either delay or inhibit the oxidisation of that substance.[8] The body uses these antioxidants as a means of defence to scavenge free radicals generated in diabetes mellitus. These

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**Table 1.** Comparison between test group and control group.

| Parameters | Test group | Control group | *P* value |
|---|---|---|---|
| Random blood glucose (mg/dL) | 197.74±49.29 | 104.79±11.33 | 0.001 |
| Glycosylated haemoglobin (HbA1c, %) | 9.86±1.44 | 5.21±1.23 | 0.001 |
| Glutathione reductase (U/L) | 45.81±20.45 | 61.21±14.34 | 0.001 |
| Total antioxidant status (mmol/L) | 1.81±1.04 | 2.73±2.08 | 0.030 |

*P*<0.05 regarded as significant.

free radicals are generated, for example, via glucose auto-oxidation and mitochondrial leaks.[9] An example of antioxidant defence systems includes antioxidant enzymes, chain breaking antioxidants and transition metal binding proteins. Depletion of antioxidant status has been implicated in the development of diabetic complications among individuals with type 2 diabetes mellitus. Although, the incidence of type 2 diabetes mellitus in females has become lower than in males,[10,11] the rate of developing diabetic complications such as heart disease and blindness is higher in women.[10] Hence, it is imperative to monitor the antioxidant status of females with type 2 diabetes.

This study aims to evaluate the total antioxidant status in relation to glycaemic control of females with type 2 diabetes mellitus attending Nnamdi Azikiwe University Teaching Hospital, Nnewi, Anambra State.

## Materials and methods

### *Participants*

This study recruited a total of 86 females. Forty-three apparently healthy females formed the control group. The test group consisted of 43 already confirmed patients with type 2 diabetes mellitus attending the out-patient diabetic clinic of Nnamdi Azikiwe University Teaching Hospital (NAUTH), Nnewi, from October 2013 to January 2014. Participants with type 2 diabetes were further subdivided based on their glycaemic control, using cut-off points as established by the American Diabetes Association,[12] into good glycaemic control (<7% HbA1c) and poor glycaemic control (>7% HbA1c). Ethical approval was obtained from the Ethics Committee, Nnamdi Azikiwe University Teaching Hospital, Nnewi. Written informed consent and a questionnaire were obtained from all participants prior to the start of the study.

### *Inclusion and exclusion criteria*

Inclusion criteria were female patients already diagnosed with type 2 diabetes mellitus by their physicians and were within the age range 30–65 years. The criteria for exclusion were patients with type 1 diabetes mellitus, end-stage renal disease, HIV/AIDS, active infection, pregnant females, chronic or acute illnesses and patients suffering from endocrine disorders other than type 2 diabetes mellitus. Hypertensives, smokers and alcoholics were also excluded to remove confounding effects on oxidative stress.

### *Blood collection*

Intravenous blood was collected aseptically from participants who gave their consent and were attending the diabetic clinic of Nnamdi Azikiwe University Teaching Hospital, Nnewi.

A total of 8 mL was collected from each participant. 1.5 mL was collected into the fluoride oxalate bottle for the analysis of random blood glucose. An additional 1.5 mL was collected into the tripotassium ethylene diamine tetraacetic acid ($K_3$EDTA) bottle for the analysis of glycosylated haemoglobin (as an index for glycaemic control). Whole blood (5 mL) was collected into a plain bottle and the serum obtained was used for the analysis of glutathione reductase (as an enzymatic antioxidant index) and total antioxidant status.

### *Analysis*

The parameters were estimated using randon blood glucose (RBG),[13] HbA1c,[14] glutathione reductase (GR)[15] and total antioxidant status (TAS).[16] Controls used for analysis were

**Table 2.** Comparing biochemical parameters of female type 2 diabetics on the basis of glycaemic control between poor glycaemic test group, good glycaemic test group and control group.

| | Random blood glucose (mg/dL) | Glycosylated HbA1c (%) | Glutathione reductase (U/L) | Total antioxidant status (mmol/L) |
|---|---|---|---|---|
| Poor glycaemic test group (*n*=35) | 200.34±50.40 | 10.23±1.33 | 40.66±15.48 | 1.80±1.08 |
| Good glycaemic test group (*n*=8) | 186.38±45.39 | 6.54±0.45 | 68.38±25.09 | 1.86±0.92 |
| Control group (*n*=43) | 104.79±11.33 | 5.21±1.23 | 61.21±14.34 | 2.73±2.08 |
| *P* value | 0.000 | 0.000 | 0.000 | 0.042 |
| Poor glycaemic test group *vs.* good glycaemic test group | 0.322 | 0.000 | 0.000 | 0.923 |
| Poor glycaemic test group *vs.* good glycaemic test group | 0.000 | 0.000 | 0.000 | 0.016 |
| Control group *vs.* good glycaemic test group | 0.000 | 0.001 | 0.248 | 0.178 |

*P*<0.05 regarded as significant.

**Table 3.** Correlation showing relationship between parameters.

| Parameters | *r* value | *P* value |
|---|---|---|
| Random blood glucose *vs.* glutathione reductase | –0.403 | 0.000* |
| Random blood glucose *vs.* glycosylated HbA1c | 0.702 | 0.000* |
| Random blood glucose *vs.* total antioxidant status | –0.260 | 0.015† |
| Glycosylated HbA1c *vs.* glutathione reductase | –0.471 | 0.000* |
| Glycosylated HbA1c *vs.* total antioxidant status | –0.170 | 0.119 |
| Glutathione reductase *vs.* total antioxidant status | 0.080 | 0.464 |

*Correlation significant at $P<0.01$ †Correlation significant at $P<0.05$

obtained from pooled serum of apparently healthy non-diabetic subjects. All sera were aliquoted into Eppendorf tubes and stored frozen at –20°C until analysed.

***Statistical analysis***

The Statistical Package for Social Sciences, version 16.0, was used for the analysis of data. Results were reported as mean±standard deviation (SD). Means were analysed using independent Student's *t*-test to check for differences between subject and control groups. Pearson's correlation coefficient (*r*) was used to determine the relationship between means of the variables. Results were regarded as statistically significant at $P<0.05$.

## Results

The test group was observed to have a significant increase in random blood glucose (RBG) and HbA1c when compared to the control group ($P<0.05$). There was significant decrease in both GR and TAS in female diabetic subjects when compared to the control group ($P<0.05$) (Table 1).

There was significant increase in RBG and HbA1c among the poor glycaemic test group, compared to the good glycaemic test group and the control group. GR was significantly increased in the good glycaemic test group when compared to the poor glycaemic test group and control group. TAS was observed to be significantly increased in the control group when compared both to the poor glycaemic test group and good glycaemic test group (Table 2).

There were significant negative correlations between RBG and GR, RBG and TAS, HbA1c and GR. This indicates that a rise in RBG and HbA1c would lead to decrease in GR activity and TAS depletion. There was a positive correlation between RBG and HbA1c; this indicates that increase in RBG concentration leads to increased HbA1c concentration (Table 3).

## Discussion

Hyperglycaemia is a common effect of uncontrolled diabetes mellitus and over time leads to serious damage to many of the body's systems, especially the nerves and blood vessels.[17] The uncontrolled presence of hyperglycaemia increases the generation of free radicals, which in turn leads to diabetic complications via increased flux through the polyol pathway (in which glucose is reduced to sorbitol, lowering levels of both reduced nicotinamide adenine dinucleotide phosphate [NADPH] and reduced glutathione); increased formation of advanced glycation end products (AGEs) and receptors (RAGE); activation of protein kinase C (PKC) (with effects ranging from vascular occlusion to expression of proinflammatory genes);[18] and increased shunting of excess glucose through the hexosamine pathway (mediating increased transcription of genes for inflammatory cytokines).

As expected, increases in both RBG and HbA1c in the test group confirm diabetes mellitus. In this group, the findings are in accordance with other reports,[19,20] where increases in RBG and HbA1c in participants with diabetes mellitus were noted. GR activity was generally reduced in diabetes; further reduction was observed in the poor glycaemic test group. Strikingly, the highest GR activity was observed in the good glycaemic test group. The reduction of GR activity among participants with diabetes is probably due to the depletion of GR in attempting to restore balance from possible oxidative stress as a result of hyperglycaemia-induced free radicals. The activation of the polyol pathway might be responsible for the further reduction in GR activity in the poor glycaemic control group.

In the diabetic state, increased flux through the polyol pathway (in which glucose is reduced to sorbitol, lowering levels of both reduced nicotinamide adenine dinucleotide phosphate [NADPH] and reduced glutathione) occurs.[18] This finding is in accordance with previous reports by Manjulata and colleagues.[21]

The increase in GR activity in the good glycaemic test group is probably due to inactivation of the polyol pathway and hence increased generation of NADPH. This finding is in accordance with work by Ehsaneh *et al*.[20] TAS was significantly lowered in participants with type 2 diabetes when compared to the control group. Further depletion was observed in the poor glycaemic test group compared to the good glycaemic test group, and the control group. This is probably due to the auto-oxidation of glucose that results in the formation of hydrogen peroxide, which is a major source of free radical production,[22] and increases oxidative stress,[18] thereby reducing antioxidant activity. A similar finding has been reported elsewhere.[23]

RBG and HbA1c both had a significant negative correlation with glutathione reductase activity. This implies that increase in RBG and HbA1c would lead to reduction in GR activity. This could be due to increased sorbitol generation from glucose via the polyol pathway, which reduces NADPH availability and glutathione reductase activity. As expected, a significant positive correlation was

observed between RBG and HbA1c. The presence of hyperglycaemia increases the non-enzymatic process of glycation. RBG had a significant negative correlation to TAS. This is probably due to a shift in equilibrium that favours oxidative stress generated from hyperglycaemic-induced glucose auto-oxidation.

This study reports that the majority of females with type 2 diabetes recruited for this study had poor glucose management, as they presented with poor glycaemic control. A reduction in GR activity and marked depletion in TAS is suggestive of possible onset of diabetes complications. Hence, it is very necessary that these parameters be recommended as routine tests in diabetic clinics as it will aid in rapid diagnosis and proper management of diabetic complications in these patients. □

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